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On the pre-adult instars of *Pseudococcus longispinus* Targ., with special reference to characters of possible generic significance (Hem.). By H. C. James, B.Sc., Ph.D. . . . . pp. 73–84

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ON THE PRE-ADULT INSTARS OF *PSEUDOCOCCUS LONGISPINUS* TARG., WITH SPECIAL REFERENCE TO CHARACTERS OF POSSIBLE GENERIC SIGNIFICANCE (HEM.)

By H. C. James, B.Sc., Ph.D.

(*From the Entomological Department, Zoological Laboratory, Cambridge.*)

[Read 18th November, 1936.]

With Six Text-figures.

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Introductory.

This paper has been written as the result of a study of the immature stages of *P. longispinus* and their comparison with numerous larvae of other species of *Pseudococcus* (*sens. str.*). Comparative studies indicate that three larval instars in the female and four in the male are usual and should perhaps be regarded as invariable for the genus *Pseudococcus*. Berlese (2) is the only entomologist who has treated the pre-adult instars of *Pseudococcus* from the morphological standpoint, and he found that there are three larval instars in the female of *P. citri* Risso and *P. longispinus* and one larval and two pupal stages in the male. It is now apparent, however, that one instar was overlooked in the male, owing to the defective staining methods then in vogue (1893) failing to disclose the fact that sexual dimorphism is recognisable in the second instar as will be hereinafter described.

Since the work of Berlese a number of biological studies of species of *Pseudococcus* have been made, and Hough (3) in *P. comstocki*; Kirkpatrick (7) in *P. lilacinus* (now re-described as *P. kenyae* Le Pelley); Myers (8) in *P. citri*; and Basinger (1) in *P. gahani* discovered three larval instars in the female and four in the male of the species with which they were concerned. However, critical morphological examination of the larval stages does not appear to have

been made, and this is almost imperative in the early instars if full assurance is desired that all stages have been observed.

As I had on hand a series of mounts of all the stages of a number of species I decided to describe the pre-adult instars of *longispinus*, the genotype of *Pseudococcus*, and by a comparative study with the corresponding stages of other species to recognise characters which I considered to be uniformly distinctive of each instar.

If the work of Uitchanco (9) on the life-history of *Trionymus sacchari* is confirmed, it suggests that studies of the pre-adult instars of species of *Pseudococcus* and of allied genera will be of assistance in the delimitation of a number of nebulous generic boundaries in this group.

I wish again to express my indebtedness to Dr. A. D. Imms, F.R.S., for facilities and useful suggestions.

(1) Oviposition and oviparity in *P. longispinus*.

The egg of *longispinus* is an ovoid pale yellow object about 0·25 mm. long, and at the time of oviposition the contained embryo is fully formed. The process of hatching begins about fifteen minutes after oviposition, and thus although the duration of external incubation is very short *longispinus* is technically speaking oviparous.

If a number of ovipositing females are placed in glass tubes so that the ventral surface of the abdomen can be kept under observation an egg will soon be detected in the act of being expelled through the genital pore. Almost immediately after oviposition movements of the egg brought about by the full-grown embryo can sometimes be seen, and microscopic observation will then reveal that the chorion is closely appressed to the contained embryo and that the outlines of the latter are apparent.

The rupture of the chorion takes place ventrally between the prothoracic legs, and is effected by vigorous movements of the rostrum after the egg membrane has been drawn taut by the flexure of the body. The prothoracic limbs are freed first and are soon followed by the antennae and the mesothoracic limbs; the liberation of the hind limbs are effected mainly by vigorous movements of the abdomen, and the exuvia is gradually worked back to the hind end of the body, where it dries and is subsequently rubbed off.

Hatching in *longispinus* was watched with more than usual interest, owing to the allegation (9) that the act of eclosion occurs *in utero* in a species of mealybug (*Trionymus sacchari*) whose ova appear to have a period of external incubation comparable with that of *longispinus*. It is held that what issues from the genital pore of *T. sacchari* are not ova but forms comparable with the vermiform larvae of Uvarov (10), and that what has hitherto been mistaken for the act of eclosion is really the first post-embryonic moult.

Hence the matter has importance in the fixation of the number of instars in *longispinus*, and it was observed in the case of the latter species that the first pellicle cast after expulsion from the vagina is a simple capsule without separate investing sheaths for the antennae and limbs which would necessarily form part of the exuvia of the first post-embryonic ecdysis.

(2) The Pre-adult instars of *P. longispinus*.

(a) *The first instar.*

The first instar at eclosion is of the same colour as the egg, and varies in length from about 0·25 mm. at hatching to about 0·60 mm. in length and

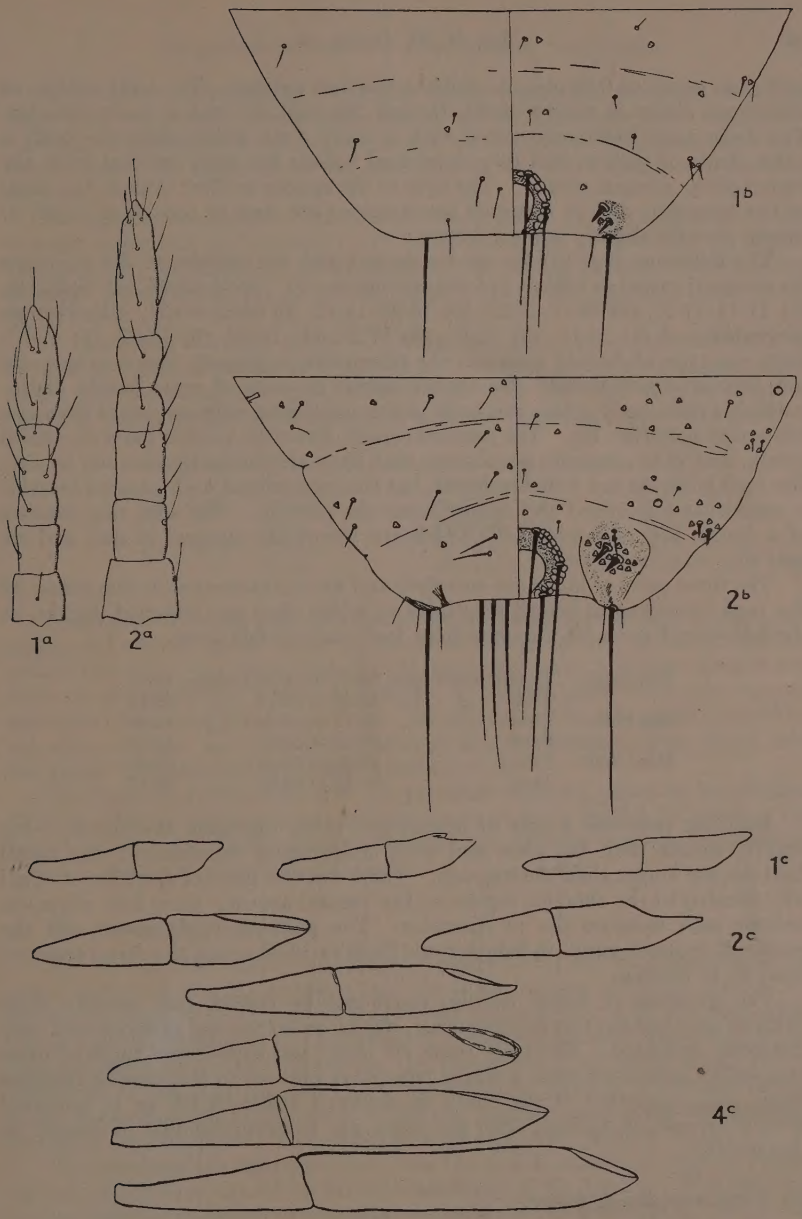


FIG. 1*a*.—The first instar. An antennae, × 150 diams. 1*b*.—The first instar. The left side represents the posterior ventral aspect of the abdomen and the right side the posterior dorsal aspect of the same, × 150 diams. 1*c*.—Outlines of the tibiae and the tarsi of the fore, mid, and hind limbs of the first instar, × 150 diams.

FIG. 2*a*.—The second instar ♀. An antenna ♀, × 153 diams. 2*b*.—The second instar. The left side represents the posterior ventral aspect of the abdomen and the right side the posterior dorsal aspect of the same, × 150 diams. 2*c*.—The second instar ♀. The outlines of the tibiae and tarsi of the fore, mid, and hind limbs, × 150 diams.

FIG. 4*c*.—The third instar ♀. The outlines of the tibiae and the tarsi of the fore, mid, and hind limbs showing relative lengths of tibiae and tarsi, × 150 diams.

0·28 mm. to about 0·90 mm. in width at the first ecdysis. The body outline as seen from above is nearly ovoid, though the cephalic end is more rounded. The derm soon becomes covered with a waxy dust which gives the body a paler shade of yellow, and two short anal tassels are soon secreted from the region of the paranal cerarii. The eyes in life appear as dark dots at the bases of the antennae, and in mounted preparations are seen to consist of a pair of simple circular slightly convex lenses.

The antennae (fig. 1*a*) are six-segmented and the lengths of the segments (in microns) varied as follows in forty specimens : (1) 20·24–23·36, (2) 20·37–23, (3) 17·11–17·27, (4) 13·17–17·28, (5) 16·98–17·12, (6) 53·23–60·31, with average proportions of (1) 22·13, (2) 22·17, (3) 17·22, (4) 16·38, (5) 17·07, (6) 57·67. Only one type of dermal waxpore, the triangular, is present, and it is sparsely but generally distributed; the cerarii consist of pairs of setae hardly distinguishable from body setae, and each pair is associated with not more than one triangular pore (fig. 1*b*). The paranal cerarii, however, possess pairs of conical spines, and it is a specific peculiarity that they are markedly unequal in size. The anal lobes are not well developed, but the area around each paranal cerarius is somewhat sclerotised and caudal setae are present. The anal ring consists of a double row of beaded cells which are somewhat unequal in size, and six anal ring setae are present.

The three pairs of limbs are complete and are characterised in this instar by the tarsi exceeding in length the tibiae to which they are attached (fig. 1*c*) as the following figures (in microns) from forty individuals show.

Fore limb.	Tibiae	varied	from	36·67 to 46·62	average	41·81
	Tarsi	,,	,,	43·36 to 56·71	,,	53·42
Mid limb.	Tibiae	,,	,,	40·13 to 46·24	,,	44·84
	Tarsi	,,	,,	50·23 to 56·58	,,	54·67
Hind limb.	Tibiae	,,	,,	40·37 to 56·28	,,	53·31
	Tarsi	,,	,,	50·32 to 63·37	,,	59·27

Each leg possesses a pair of ungual and tarsal digitules, the former being slightly longer than the claw and with club-shaped extremities; the tarsal digitules are longer than the unguals. There are two pairs of spiracles of equal size situated in the thoracic region on the ventral aspect; their four stigmatic orifices each measure 5 μ in diameter. The rostrum is dimerous and the proximal segment varies in length from 13·29 to 16·65 μ and the distal segment from 30 to 33·34 μ.

The presence of dorsal ostioles could not be traced, but possibly their outlines were too faint to be recognised, but an ovoid ventral cicatrice was very distinctly indicated. The body setae are short and sparse, and most of them are closely associated with a single triangular pore as in the case of cerarian setae. No indication of sex could be detected either in life or in mounted specimens, and it appears that the sexes are indistinguishable externally in this instar.

(b) *The second female instar.*

In life the second larval instar differs little externally from the first instar apart from the fact that the waxy coating is somewhat denser, the anal tassels are longer and a few long glassy filaments of wax project here and there from the body surface. The measurement of mounted preparations shows that the size varies from 0·60 mm. to 0·96 mm. in length and from 0·30 to 0·40 mm. in maximum width.

Although the antennae of this instar are six-segmented as in the preceding stage they can readily be distinguished by the fact that the third segment is markedly longer than either segments four or five (fig. 2*a*), whereas the third, fourth and fifth segments of an antennae of the first instar are equal or subequal.

The antennal segments of the second female instar vary in length as follows in forty specimens (in microns): (1) 26·62–33·12, (2) 23·27–33·33, (3) 30·21–36·71, (4) 20·18–23·42, (5) 23·48–26·12, (6) 63·25–70·11, with the following average proportions, (1) 29·54, (2) 28·62, (3) 33·45, (4) 22·67, (5) 25·36, (6) 67·82.

In this instar the tarsi instead of being markedly longer than the tibiae as in the first instar are equal or subequal to the tibiae in the prothoracic pair of limbs, and the tibiae are appreciably longer than the tarsi only in the hind pair (fig. 2*c*), as the following figures (in microns) from forty specimens show.

Fore limb.	Tibiae varies	from	63·35	to	70·47	average 65·43
	Tarsi ,,	,,	63·30	to	70·52	,, 65·24
Mid limb.	Tibiae ,,	,,	66·79	to	73·24	,, 69·71
	Tarsi ,,	,,	63·56	to	70·13	,, 67·45
Hind limb.	Tibiae ,,	,,	80·24	to	86·78	,, 84·37
	Tarsi ,,	,,	73·38	to	80·41	,, 78·73

There are seventeen pairs of cerarii, the number characteristic of the adult stage, and conical spines occur on the penultimate as well as on the paranal cerarii (fig. 2*b*); the triangular dermal waxpores have a denser distribution than in the preceding instar, and there is always more than one waxpore associated with each paranal cerarius. In addition to the triangular pores there are also present a few cylindrical ducts, 3·33 μ in diameter, from which arise the glassy filaments of wax already referred to.

The rostrum is dimerous and the proximal segment varies in length from 13·32 to 16·60 μ, and the distal segment from 43·33 to 46·66 μ in length. The dorsal ostioles are clearly demarcated and the ventral cicatrice is similar in shape to that of the preceding instar.

(c) *The second male instar.*

The second male instar immediately before it begins the formation of the pupal cocoon is longer, narrower, and the body content is darker than a full-grown specimen of the corresponding instar of the female. In mounted specimens, however, owing to the flattening they usually undergo and owing to the disappearance of the body contents, the separation of the sexes would not be easy were it not that the male has a considerable number of circular dermal pores, measuring 6 μ in diameter, which are peculiar to that sex and are most probably concerned with the elaboration of the pupal cocoon (fig. 3).

The antennae are six-segmented as in the female and their relative lengths do not depart to a significant extent from those of the female. The following is the variation in length (in microns) exhibited by 40 individuals: (1) 26·72–31·29, (2) 26·61–30·42, (3) 32·97–33·14, (4) 16·62–23·24, (5) 20·12–26·52, (6) 61·66–63·24, and the average lengths are (1) 28·22, (2) 28·81, (3) 33·05, (4) 21·25, (5) 23·42, (6) 62·62.

The relative lengths of the tibiae and tarsi approximate fairly closely to those of the female, but the tibiae are rather longer in relation to the tarsi, as the following figures (in microns) show.

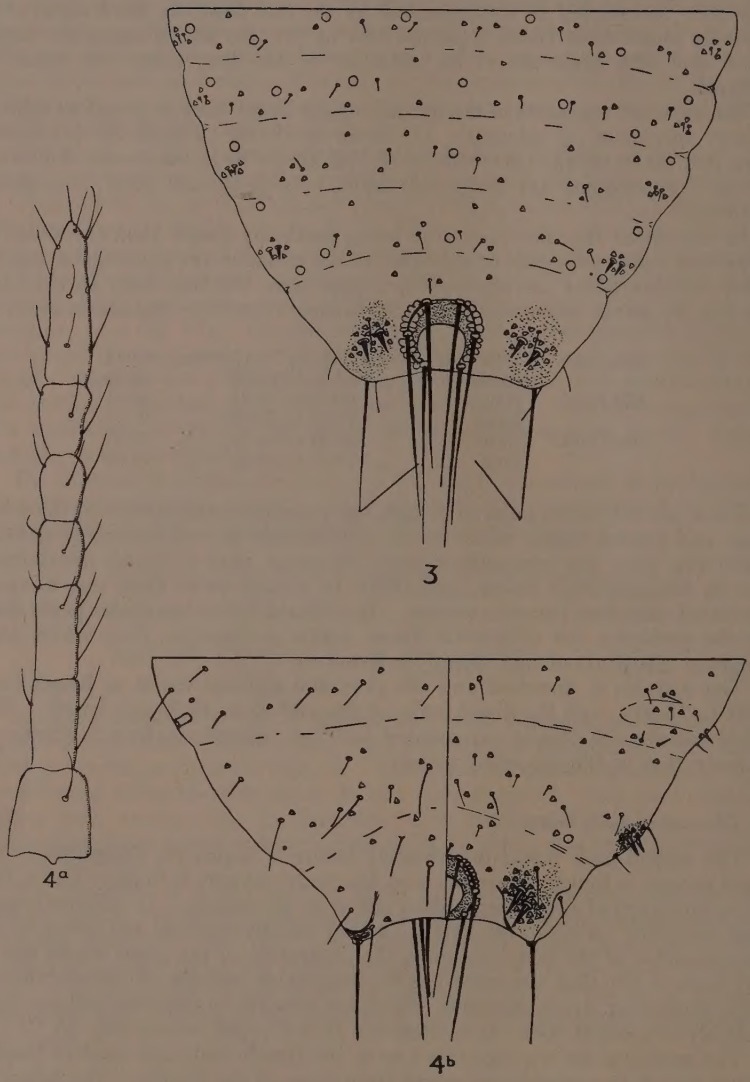


Fig. 3.—The second instar ♂. The posterior dorsal aspect of the abdomen × 150 diams.
Fig. 4*a*.—The third instar ♀. An antenna, × 150 diams.
4*b*.—The third instar ♀. The left side represents the posterior ventral aspect of the abdomen and the right side the posterior dorsal aspect of the same, × 150 diams.

Fore limb.	Tibiae	varies from	66·62	to	71·40	average 68·50
	Tarsi	,, ,,	61·24	to	67·77	,, 66·47
Mid limb.	Tibiae	,, ,,	67·34	to	71·57	,, 70·53
	Tarsi	,, ,,	65·74	to	69·10	,, 66·38
Hind limb.	Tibiae	,, ,,	83·54	to	87·95	,, 86·67
	Tarsi	,, ,,	67·37	to	72·42	,, 70·53

The length of full-grown specimens varies from 0·99 mm. to 1·12 mm.

(d) *The third female instar.*

The external appearance of the third female instar is very similar to that of a young adult. The marginal tassels are well developed and they increase in length posteriorly, with the hindmost pair markedly longer as in the adult. In life the third female instar can be distinguished from the young adult under the microscope by its shorter and stouter antennae. Usually it is not possible to count the segments of the antennae in life owing to the debris adhering to them.

The antennae are seven-segmented and their lengths (in microns) vary as follows in forty specimens: (1) 43·33–46·62, (2) 40·11–47·73, (3) 40·37–47·67, (4) 26·27–36·38, (5) 30·62–36·19, (6) 30·28–40·32, (7) 73·47–86·28, with average lengths of (1) 44·37, (2) 44·33, (3) 45·12, (4) 32·02, (5) 33·21, (6) 35·27, (7) 80·24 (fig. 4*a*).

There are two pairs of ventral thoracic spiracles and the anterior pair is smaller as its peritremes vary from 10 to 12 μ in diameter, whilst those of the posterior pair vary from 12 to 14·3 μ.

The rostrum is dimerous and its proximal segment measures from 30 to 40·24 μ and its distal segment from 76·25 to 90·00 μ. The cerarii are even better demarcated than in the preceding instar, the cerarian spines being stouter and the aggregations of associated pores larger; furthermore, dermal pores both circular and triangular are more numerous and the dermal vestiture longer and fuller (fig. 4*b*).

The seven-jointed antennae, the absence of the genital pore and its attendant circular multilocular dermal pores clearly distinguish this instar from adult females. The third female instar varies in length from 0·90 mm. to 1·20 mm.

(e) *The third male instar or " prepupal " stage.*

After the pupal cocoon is almost completed an ecdysis occurs and a pupiform creature is revealed whose principal characteristics are a small pair of external wing-buds in the mesothoracic region, the absence of mouth-parts and a true anal ring (fig. 5).

Although the climb claws are relatively long and slender and digitules are absent the limbs are capable of slow perambulation. Triangular dermal pores which are present in the two preceding stages of the male and in all the stages of the female, are absent. The characteristic dermal pore is circular and 5·6 μ in diameter; there are also tubular dermal ducts of two-size categories which measure 3·6 μ and 2·6 μ respectively. The body vestiture is short and sparse. The " prepupal " stage varies from 0·90 mm. to 1·20 mm. in length.

(f) *The fourth male instar or " pupal " stage.*

The fourth male instar is characterised and easily recognised by the pair of elongated wing-cases into which the wing-buds of the previous instar have grown. The absence of mouth-parts, triangular dermal pores and anal ring

are also features of this instar (fig. 6). Circular pores of a pattern and calibre similar to those of the third male instar are present, as are also a number of tubular ducts measuring 3·60 μ.

The " pupa " is capable of slow movement, but the tarsi and claws are relatively long and slender, only slightly curved and without digitules. Both " pupa " and " prepupa " are capable of secreting new cocoons if removed from the old ones.

(3) Characters common to the pre-adult instars of *Pseudococcus* spp. (*sens. str.*).

(a) *The first instar.*

The examination of the first instar forms of a considerable number of species of *Pseudococcus* (4, 5, and 6) and their comparison with those of *longispinus*, the genotype, showed the following characters to be distinctive of the first instar forms of *Pseudococcus* spp. (*sens. str.*).

(1) The tarsi of all three pairs of limbs longer than the tibiae to which they are attached.

This character is plainly evident in all the very numerous first-instar forms examined by me. The figures indicating the relative length of tibiae and tarsi have already been given for *longispinus* (sect. 2*a*), and similar measurements (in microns) are here given for the species *P. citri* Risso and *P. lilacinus* Ckll.

P. citri (40 specimens).

Fore limb.	Tibiae	varies	from	40·34 to 43·36	average	42·38
	Tarsi	,,	,,	46·77 to 53·91	,,	50·73
Mid limb.	Tibiae	,,	,,	40·57 to 46·68	,,	44·21
	Tarsi	,,	,,	50·78 to 53·49	,,	51·23
Hind limb.	Tibiae	,,	,,	46·83 to 53·80	,,	49·61
	Tarsi	,,	,,	55·31 to 57·12	,,	56·14

P. lilacinus (40 specimens).

Fore limb.	Tibiae	varies	from	40·72 to 43·35	average	41·32
	Tarsi	,,	,,	46·61 to 50·81	,,	52·19
Mid limb.	Tibiae	,,	,,	40·17 to 46·38	,,	42·29
	Tarsi	,,	,,	50·21 to 53·96	,,	52·17
Hind limb.	Tibiae	,,	,,	50·72 to 53·28	,,	51·52
	Tarsi	,,	,,	56·23 to 60·71	,,	57·86

(2) The triangular-shaped pore is the only type of dermal waxpore present.

(3) The antennae are six-segmented and segments three, four and five are equal or subequal.

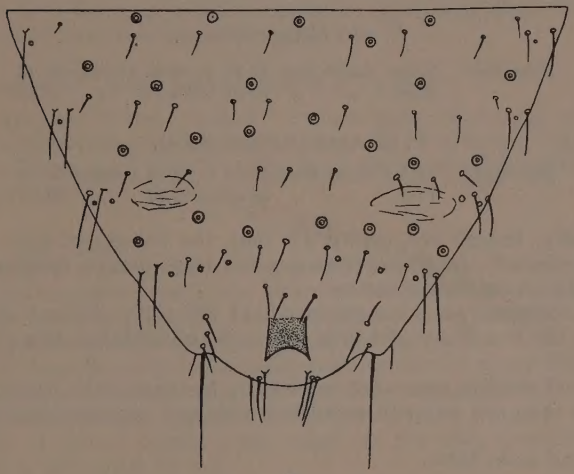
(4) Conical cerarian spines confined to the paranal cerarii.

(5) Not more than one waxpore associated with each cerarius.

(b) *The second female instar.*

The following characters are common to the second female instar forms of *Pseudococcus* spp. (*sens. str.*).

(1) The tibiae and tarsi of each pair of limbs do not differ markedly in length. Usually they are equal or subequal in the prothoracic pair and the tibiae are only slightly longer than the tarsi in the hinder pairs.



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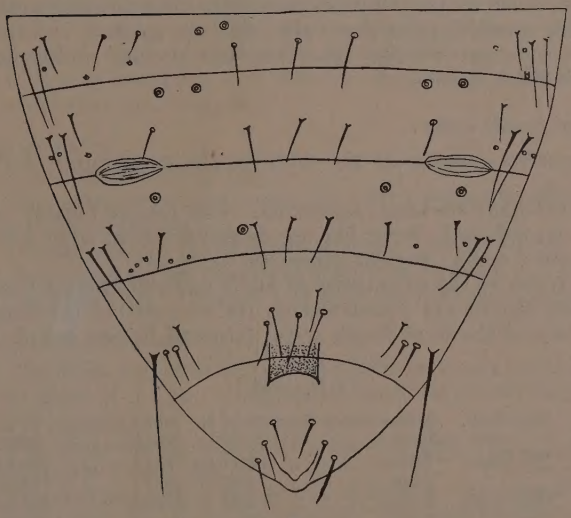

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FIG. 5.—The third instar ♂ or the "prepupa" showing the dorsal aspect of the abdomen, × 140 diams.

FIG. 6.—The fourth instar ♂ or the "pupa" showing the dorsal aspect of the abdomen soon after the "pupal" ecdysis, × 140 diams.

The measurement of the fore limbs of *P. citri* and *P. lilacinus* resulted as follows :—

P. citri (40 specimens).

Fore limb.	Tibiae	varies from	53·24	to 56·37	average 55·73
	Tarsi	,, ,,	56·37	to 61·39	,, 57·29

P. lilacinus (40 specimens).

Fore limb.	Tibiae	varies from	53·71	to 60·24	average 58·10
	Tarsi	,, ,,	58·39	to 63·47	,, 60·73

(2) Usually, though not invariably, only the triangular type of dermal waxpore is present. In *P. longispinus* a few large dermal tubular ducts are present in the second female instar.

(3) The antennae are six-segmented and the third segment is markedly longer than the fourth and the fifth segments, which latter two are equal or subequal.

(4) Conical cerarian spines not confined to the paranal pair of cerarii.

(5) More than one waxpore associated with each paranal cerarius.

(c) *The second male instar.*

The second male instar of *Pseudococcus* can easily be distinguished from the corresponding stage in the female by the possession of a number of special dermal pores, which are probably concerned with the production of the " pupal " cocoon. This special type of pore varies with the species; in *longispinus* it is of the open-mouthed circular type, but the circular multilocular type is commoner in other species.

(d) *The third female instar.*

The characters common to the third female instar forms of *Pseudococcus* are :—

(1) The antennae are seven-segmented. This feature reliably distinguishes third- from second-instar forms but not necessarily from adult females, which may have either seven- or eight-segmented antennae.

(2) The tibiae of the three pairs of limbs markedly longer than the tarsi. The following figures are measurements (in microns) of the lengths of the tibiae and tarsi of the third female instar forms of *P. citri* and *P. lilacinus*.

P. citri.

Fore limb.	Tibiae	varies from	76·32	to 80·52	average 77·25
	Tarsi	,, ,,	70·17	to 76·40	,, 72·42
Mid limb.	Tibiae	,, ,,	83·41	to 93·12	,, 90·41
	Tarsi	,, ,,	73·57	to 83·71	,, 77·56
Hind limb.	Tibiae	,, ,,	96·51	to 106·82	,, 102·49
	Tarsi	,, ,,	80·47	to 90·14	,, 87·61

P. lilacinus.

Fore limb.	Tibiae	varies from	76·66	to 86·12	average 80·45
	Tarsi	,, ,,	60·17	to 73·21	,, 67·34
Mid limb.	Tibiae	,, ,,	86·62	to 90·53	,, 88·37
	Tarsi	,, ,,	73·78	to 83·41	,, 76·85
Hind limb.	Tibiae	,, ,,	100·00	to 106·72	,, 103·72
	Tarsi	,, ,,	80·45	to 86·34	,, 84·57

(3) The absence of the genital pore and its attendant circular multilocular dermal pores is the most reliable character separating third-instar females of *Pseudococcus* from young adult females.

(e) *The " prepupal " and " pupal " instars of the male.*

The " prepupal " and " pupal " instars agree in lacking mouth-parts, triangular dermal pores and a true anal ring. The " prepupa " can always be distinguished from the " pupa " by the much greater development of the wing-cases on the latter.

Summary.

(1) The study of the pre-adult stages of *P. longispinus* Targ., including the egg and their comparison with the immature stages of many other species of *Pseudococcus* (*sens. str.*), has led to the conclusion that three larval instars in the female and four in the male are typical for the genus.

(2) In *P. longispinus* eclosion takes place externally about fifteen minutes after oviposition. The sexes are indistinguishable externally in the first instar, but sexual dimorphism is apparent in the second instar owing to the presence in the male of special dermal pores which are probably concerned with the elaboration of the pupal cocoon.

The external morphology of the pre-adult instars is described, and numerous measurements showed that such characters as the relative lengths of tibiae and tarsi, the relative lengths of the antennal segments, the diameter of the peritremes of the spiracles, and the relative lengths of the segments of the rostrum vary within limits characteristic for each instar.

(3) The following characters were found to be distinctive for the pre-adult stages of *Pseudococcus* spp. (*sens. str.*).

The first instar :—

(*a*) Tibiae shorter than tarsi.

(*b*) The antennae six-segmented in which segments three, four and five are equal or subequal.

(*c*) Conical cerarian spines confined to the paranal cerarii.

(*d*) Only one waxpore associated with each cerarius.

(*e*) Only one type of dermal waxpore, the triangular, is present.

(4) The second female instar :—

(*a*) The tibiae and tarsi of the prothoracic limbs are equal or subequal, and the tibiae of the two hind pairs of limbs not greatly longer than the tarsi.

(*b*) The antennae six-segmented and the third segment is markedly longer than the fourth and fifth.

(*c*) The conical cerarian spines not confined to the paranal cerarii.

(*d*) More than one waxpore associated with each paranal cerarius.

(*e*) Usually though not invariably only one type of dermal waxpore, the triangular, is present.

(5) The third female instar has

(*a*) The antennae seven-segmented.

(*b*) The tibiae of all three pairs of limbs markedly longer than the tarsi.

(*c*) The genital pore and its attendant circular multilocular pores are absent.

(6) The "prepupal" and "pupal" stages are without mouth-parts, triangular dermal pores and a true anal ring.

Small mesothoracic external wing-buds are present in the "prepupa" and elongated wing-cases in the "pupa."

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